

THE CELLULAR STRUCTURE OF LYMPHOMYELOID TISSUES IN *CHIMAERA MONSTROSA* (PISCES, HOLOCEPHALI)

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ABSTRACT

The ultrastructure of the main lymphomyeloid organs of the holocephalan fish, *Chimaera monstrosa*, is presented.

The thymus is well developed even in adults. It is densely packed with small and large lymphocytes, the former predominating. The ultrastructure of *C. monstrosa*'s lymphocytes is similar to that of mammalian ones. The lymphocytes show intimate connections with large epithelial reticulocytes, which infiltrate the cytoplasm of the lymphocytes with long processes.

The lymphomyeloid tissues around the orbit are mainly granulocytopenic. Two types of granulocytes occur: eosinophilic and heterophilic. The latter is found in excess. The granulocytes are ultrastructurally similar to those of elasmobranchs such as *Etmopterus spinax* and have similar characteristic granules. In addition to granulocytes in various stages, lymphocytes, plasma cells, blast cells, and solitary macrophages are scattered throughout the tissue.

The suprapalatal region also has a similar supply and variety of cells, but most heterophilic granulocytes have modified granules.

In the spleen, erythrocytes in different developmental stages are common. This confirms earlier studies which suggested that the spleen functions primarily as an erythrocytopenic organ.

INTRODUCTION

In the mid-nineteenth century, Leydig (1851, 1857) described lymphoid tissue in the orbital and in a suprapalatal region of a holocephalan fish, *Chimaera monstrosa*. The presence of lymphomyeloid tissues in the head of holocephalans has been noticed by a few other authors. Kolmer (1923) investigated such tissue in the suprapalatal region and ascribed it a bone marrow-like function. Holmgren (1942) briefly described the distribution of lymphoid tissues in the craniums of *Chimaera* and *Hydrolagus*. Fänge and Sundell (1969) later studied the histology of these tissues in *Chimaera*. They showed that the cranial lymphomyeloid tissues of *Chimaera* contain aggregates of granulocytes, lymphocytes, and developmental stages of these cell types. Stahl (1967) reported the occurrence of a similar lymphomyeloid tissue in the shoulder region and noticed a close association of this tissue with blood sinuses. A histological study of the spleen of *Chimaera* was performed by Scatizzi (1932) who reported that the less developed white portions were lymphocytopenic and that the red parts produced granulocytes and erythrocytes.

Previous studies used light microscopy only. The present investigation shows the ultrastructure of the lymphomyeloid organs of *Chimaera monstrosa*. The organs are discussed according to the following designations: the orbital tissue including the pre-

orbital and suprapalatal tissue, the thymus, and the spleen. Probably lymphomyeloid functions are not confined to these organs but also occur in, for example, intestinal regions. However, from a morphological point of view the tissues mentioned are the most predominant. As far as we know, no previous ultrastructural studies have been performed on these tissues.

MATERIALS AND METHODS

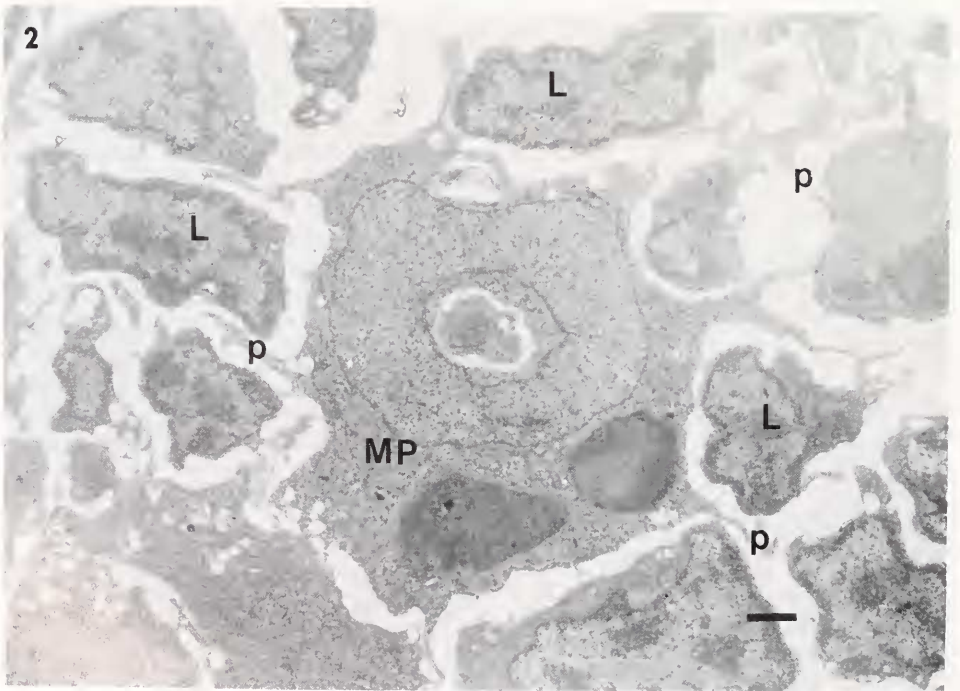
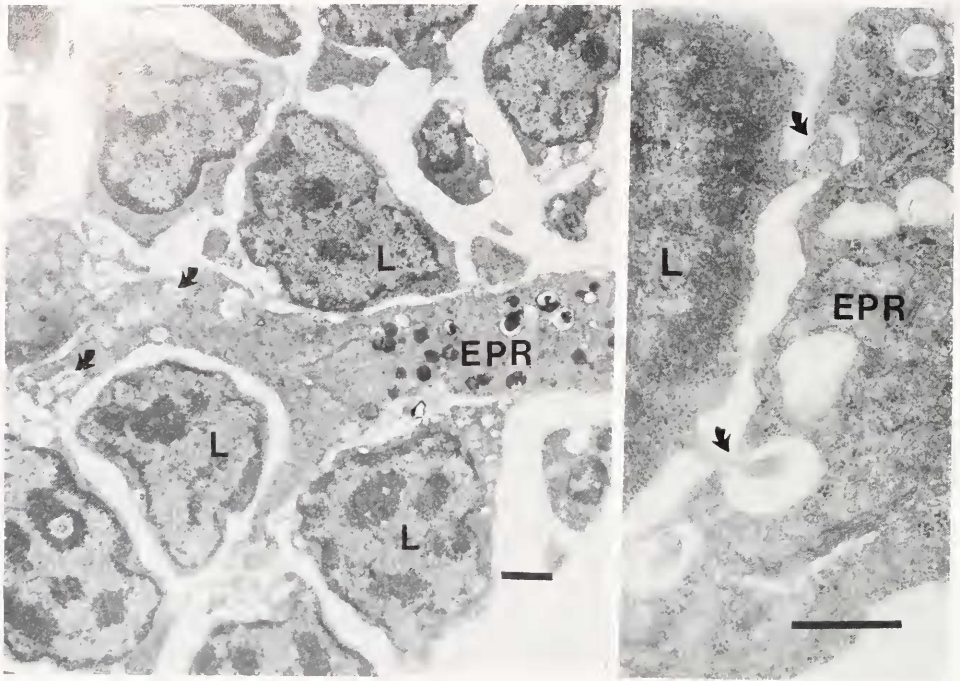
About 20 specimens of the holocephalan *Chimaera monstrosa* were caught by trawling at depths between 100 and 500 m in the region of Skagerrak to the north of Jutland, Denmark, and in a region about 10 miles to the west of Lysekil on the Swedish west coast. Samples were fixed on board the ship 10–20 minutes after the fish were captured.

In preparation for electron microscopy, samples were prefixed in 3% glutaraldehyde for 1–4 days. The glutaraldehyde was dissolved in a mixture of seawater and veronal acetate buffer, 2:1, and adjusted to neutral pH with NaOH. For some samples the glutaraldehyde solution was supplied with sucrose to a final concentration of 10% and a trace of CaCl_2 was added (*cf.*, Bell *et al.*, 1969). After washing 3–5 times in the mixture used as solvent for glutaraldehyde, the samples were postfixed in 1% OsO_4 in 0.1 M veronal acetate buffer pH 7.4. Dehydration in increasing concentrations of ethanol was followed by embedding in Epon 812. Sections were cut with an LKB ultratome and contrasted with uranyl acetate and lead citrate. The sections were observed and photographed in an Hitachi HS-8 or in a Zeiss 109 electron microscope.

RESULTS

The cells of the various lymphomyeloid tissues of *Chimaera monstrosa* have light microscopic characteristics which enable investigators to compare them with corresponding cells of other vertebrates (Fänge and Sundell, 1969). The same relationship exists with regard to the ultrastructure of these cells. Most evident similarities exist when comparing the lymphomyeloid cells of *Chimaera* with those of the elasmobranchs, *Ginglymostoma cirratum* and *Etmopterus spinax*, described previously (Fänge and Mattisson, 1981; Mattisson and Fänge, 1982).

Thymus. The anatomy of the thymus has been described previously by Hammar (1912) and by Fänge and Sundell (1969). It consists of medulla and cortex and undergoes only slight age involution. It lies in the roof of the pharynx close to the dorsal parts of the gill arches, posterior to the suprapalatal lymphomyeloid tissue and well separated from it by connective tissue. The thymus is densely packed with lymphocytes with their characteristic, small cytoplasmic volume. Together with the lymphocytes there are large irregular cells of type macrophages as well as interdigitating cells or epithelial reticulocytes (Fig. 1, 2). Lymphocytes can be small or large, however small lymphocytes predominate. Intermediates between these two lymphocytes exist and might be medium-sized lymphocytes or large ones sectioned in a non-equatorial plane. The lymphocyte nuclei possess a large amount of heterochromatin mostly located adjacent to the nuclear envelope. The nuclei are mostly non-lobed, roughly spherical, and supplied with distinct nucleoli. A thin rim of cytoplasm covers the nuclei and the small lymphocytes have a very small proportion of cytoplasm. The cytoplasm is moderately electron-dense and abundant in free ribosomes. The large lymphocytes often have pseudopodia and peripheral electron-lucent vesicles which indicate an endocytotic activity. Both types of lymphocytes often come into contact with epithelial reticulocytes (Fig. 1). The interdigitating processes of these cells sur-



round a number of lymphocytes and the latter often pierce the peripheries of the epithelial reticulocytes. The extensive surface connection between the two types of cells does not cause ultrastructural changes except for an increase of pseudopodia in the border areas. The cytoplasm of the epithelial reticulocytes is supplied with bodies having a strongly electron-dense interior. Within a section, the epithelial reticulocytes might exceed lengths of $100\ \mu\text{m}$. Macrophages containing various kinds of inclusions are scattered in the thymus (Fig. 2).

The orbital and the preorbital tissues. A whitish, soft tissue surrounds the eyes and, to a large extent, fills up the preorbital (ethmoidal) canal of the cranium. This tissue is mainly granulocytopoietic; the granulocytes constitute about 80% of all the cells. The granulocytes occur in two principal forms. Their light microscopic appearance and their staining characteristics suggest that they are eosinophilic and heterophilic (neutrophilic). The ultrastructure of the granules supports this classification. Among the granulocytes, the heterophils constitute more than $\frac{4}{5}$ of the total number. The two forms show a heterogeneous distribution.

The eosinophils (Fig. 3) have a roughly spherical form and are up to $15\ \mu\text{m}$ in diameter. The eccentric nucleus is non-segmented, mainly euchromatic. In the cytoplasm, there are layers of rough endoplasmic reticulum, which often occur peripherally and run parallel to the cell membrane. The granules structurally resemble those of the eosinophils of an elasmobranch, *Etmopterus spinax* (Mattisson and Fänge, 1982), *i.e.*, they form strongly and homogeneously electron-dense spheres up to $1.5\ \mu\text{m}$ in diameter and are bordered by a membrane (Fig. 4). As shown for *Etmopterus*, the eosinophilic granules often have a halo of different thickness. The halo is most evident close to the well-developed Golgi zone. Sometimes the halo is separated from the dense interior by a zone with an intermediate electron density (Fig. 3), but usually there is an abrupt border between the dense center and the completely electron-lucent periphery (Fig. 4). Some rod-like granules also occur.

The heterophils are somewhat smaller than the eosinophils, up to $13\ \mu\text{m}$ in diameter, and have more irregular shapes. The nuclei are slender and lobed, often horseshoe shaped, with marginal heterochromatin (Fig. 5). The nucleus form is often highly irregular (Fig. 6). A small number of mitochondria are scattered within the cytoplasm. Electron-dense dots indicating calcium deposits often occur within the mitochondria. A well-developed Golgi apparatus probably supplies the cells with the granules which are scattered throughout the cytoplasm. The granules show a great diversity within the same cell (Fig. 5). Some appear like rods but most often they are ovoid with lengths between 0.5 and $1\ \mu\text{m}$. Several granules contain a well demarcated spheric "empty" region which may occupy almost the entire granule. Similar "empty" vesicles are common in the peripheries of the cells. The dense zones of the granules often show great variations in appearance. Sometimes the dense zones are

FIGURE 1. Portion of thymus. An epithelial reticulocyte (EPR) makes close contacts with several lymphocytes (L). At the border regions, projections and inwards bents (arrows) facilitate a probable interchange. Areas with strongly electron-dense inclusions are common in the cytoplasm of the epithelial reticulocyte. Fixation: 3% glutaraldehyde in a neutral solution of $\frac{2}{3}$ seawater and $\frac{1}{3}$ veronal acetate, supplied with sucrose and Ca^{++} . Postfixation in 1% OsO_4 in veronal acetate buffer. Bars: $1\ \mu\text{m}$.

FIGURE 2. A macrophage (MP) and some lymphocytes (L) in the thymus. Two cells are engulfed and others are surrounded by long projections (p) from the macrophage. The center of the macrophage appears pierced by a portion of a cell. Fixation as in Figure 1 but without sucrose and Ca^{++} added. Bar: $1\ \mu\text{m}$.

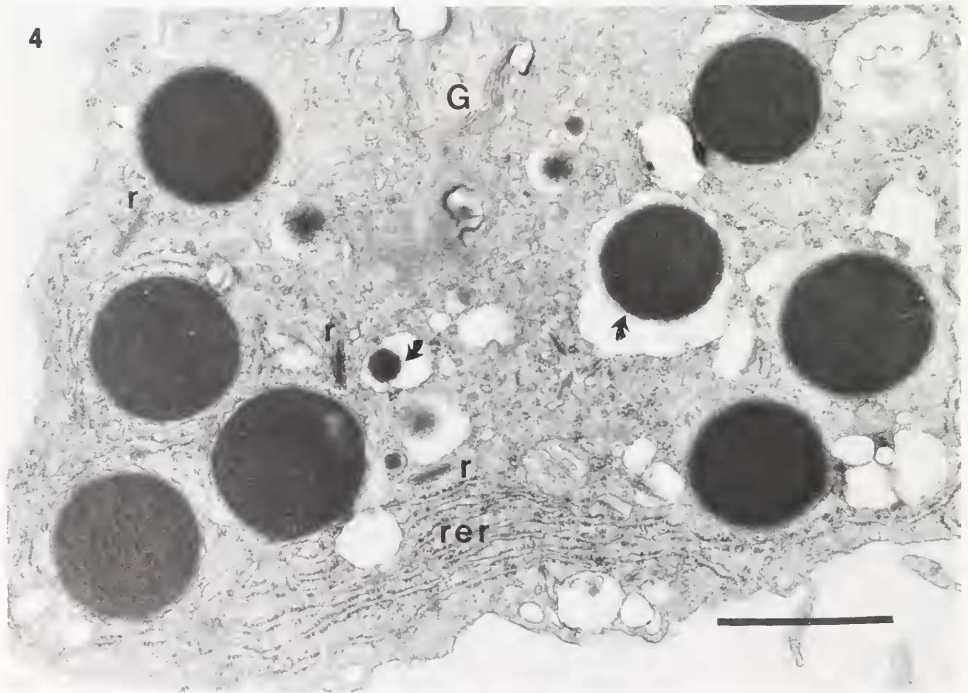
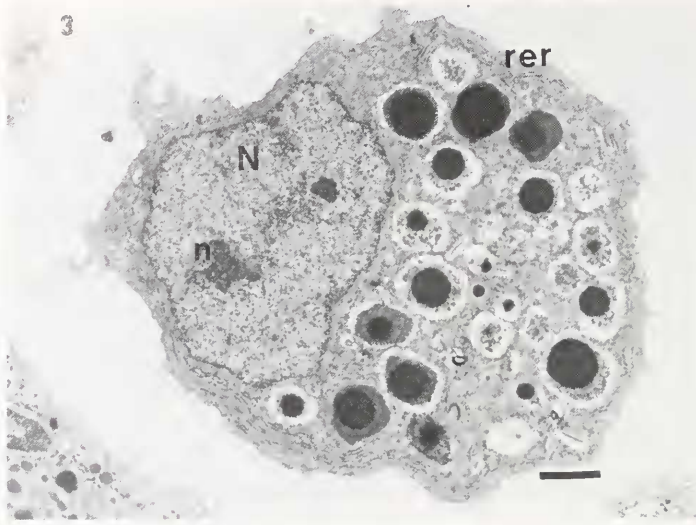


FIGURE 3. Eosinophil from the orbital region. A peripheral nucleus (N) with a nucleolus (n). A peripheral rough endoplasmic reticulum (rer). The spherical cell has spherical granules with the density increasing towards the centers, sometimes a layered density. Fixation as in Figure 2. Bar: $1 \mu\text{m}$.

FIGURE 4. Portion of an eosinophil from the orbital organ. Strictly spherical and strongly electron-dense granules supplied with membranes. Close to the Golgi complex (G), there are small introductory stages of granules. An abrupt transition in density occurs in some granules (arrows). In the cytoplasm a peripheral rough endoplasmic reticulum (rer) and some rod-like inclusions (r). Fixation as in Figure 1. Bar: $1 \mu\text{m}$.

filamentous or granular but they may also have an interior marked dense area, within which the "empty" region occurs (Fig. 7).

Intermingled with the granulocytes there are aggregations of small lymphocytes with a similar appearance to those of the thymus, *i.e.*, large dense nuclei and thin margins of cytoplasm. Larger cells which appear like large lymphocytes, monocytes, or promyelocytes are few. They contain 2–6 dense granules in each section. The granules are round, about $0.3\ \mu\text{m}$ in diameter, and have a thin marked halo and slightly undulated outlines (Fig. 8). Their ultrastructure agrees with that of granules described as azurophilic (*cf.*, Bessis, 1973).

In certain areas of the orbital tissue there are small groups of plasma cells. The plasma cells constitute less than 5% of all the cells. They are up to $15\ \mu\text{m}$ in length and often show margins that are undulated (Fig. 9a). Their cytoplasm is filled with the characteristic rough endoplasmic reticulum forming vesicular structures with a faintly electron-dense interior (Fig. 9b). The comprehensive rough endoplasmic reticulum together with the well-developed Golgi zone and the large nucleolus are all indications of an extensive protein production of these cells. Some lysosome-like bodies as well as mitochondria intermingle with the endoplasmic reticulum. In accordance with what was shown for the plasma cells of *Etmopterus spinax* (Mattisson and Fänge, 1982) the heterochromatin of *Chimaera* plasma cells does not show an arrangement like the spokes of a wheel, said to be characteristic of vertebrate plasma cells (*cf.*, Bessis, 1973).

Together with the fully developed cells are blast cells, most of them probably granuloblasts. Solitary macrophages also occur.

The suprapalatal lymphomyeloid tissue. This tissue lies in the roof of the mouth in the median line at the level of the anterior gills. It is situated in a triangular depression in the cartilage forming the base of the cranium.

As in the orbital tissue, granulocytes are the predominant cell type within the suprapalatal tissue and constitute at least $\frac{4}{5}$ of the cells. A small portion of them are eosinophils. The organelles of these cells have an ultrastructure in accordance with that of the eosinophils of the orbital tissue. Most of the granulated cells are heterophilic with their cytoplasm filled with smaller granules. However, most of the heterophils differ markedly from those of the orbital tissue. The granules are spherical and rather small, only about $0.5\ \mu\text{m}$ in diameter (Fig. 10). They are often faintly electron-dense with a non-homogeneous and variform interior and they lack the central "empty" region characteristic of heterophils from the orbital organ. The nuclei often show a low electron density. But like the heterophils of the orbital tissue, the nuclei are lobed and mostly exhibit a horseshoe-like shape.

Within some areas of the suprapalatal tissue aggregations of small lymphocytes are interspersed among the granulocytes. There are also solitary clusters of plasma cells.

The spleen. The spleen in *Chimaera monstrosa* comprises about 1% of the body weight (Fänge, 1977). A mosaic of red and white areas observed on the surface might be interpreted as an occurrence of red and white pulp. In histological sections, lymphoid areas are found immediately under the connective tissue capsule and in small numbers within the parenchyma. These cell masses have diffuse outlines and can hardly be compared with the white pulp (Malpighian bodies) found in the mammalian spleen. Previous light microscopic studies of the structure and the cell contents of the *Chimaera* spleen made by Scatizzi (1932) and by Fänge and Sundell (1969) stress the great number of erythrocytes in different developmental stages, which suggests that the spleen is an important erythropoietic organ. The same conclusion also can be drawn from the large number of erythrocytes and erythroblasts in mitosis

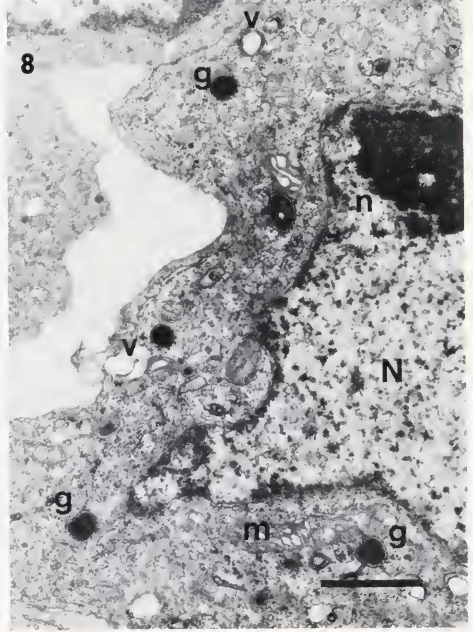
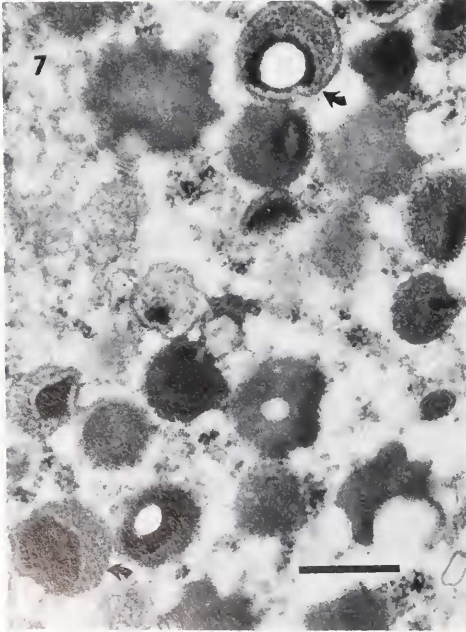
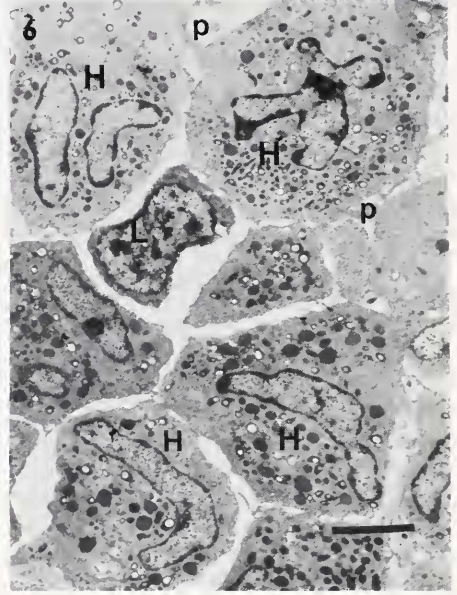


FIGURE 5. Heterophil from the orbital organ. Horseshoe-shaped nucleus (N) with marginal heterochromatin. A great diversity in the appearance of the cytoplasmic granules, several of them with electron-lucent central areas. Solitary dense rods (r), mitochondria (m) and pseudopodia (p). Fixation as in Figure 1. Bar, 1 μ m.

FIGURE 6. Low magnification of a portion of the orbital organ, showing heterophils (H) and one single lymphocyte (L). The diversity in structure of the granules and in the form of the nuclei is obvious.

found in the areas studied by electron microscopy (Fig. 11). The leucocytes constitute a minority. Among the leucocytes those of monocyte type predominate but there are also blast cells, granulocytes, and macrophages. The monocytes have their sparse, characteristic granules about $0.3 \mu\text{m}$ in length and with a thin halo. Macrophages mostly have cytoplasmic remnants of engulfed cells. No plasma cells were observed.

DISCUSSION

In Elasmobranchii, as well as in the holocephalan fish, *Chimaera monstrosa*, most lymphomyeloid tissues are located in regions unlike those of higher vertebrates (Fänge, 1984). In elasmobranchs the major lymphomyeloid tissues are found in the oesophagus and/or constitute an epigonal organ. In *Chimaera* similar tissues are located within cavities of the cranial skeleton. The majority of the cells of the lymphomyeloid tissues of *Chimaera* are structurally and ultrastructurally similar to leukocytes of higher vertebrates and a corresponding classification and terminology of the cells can be used. The high diversity in cell equipment and the occurrence of several morphological points of concordance with higher vertebrates indicate that *Chimaera* has developed a variety of immunological functions. For elasmobranchs this has been demonstrated experimentally by Good *et al.* (1966), Hildemann (1970), Sigel (1974), and Litman *et al.* (1976), among others.

The thymus of *Chimaera* is well developed and is equipped with cells similar to those found in the thymus of higher vertebrates. In addition to the predominating small lymphocytes, the thymus contains large cells of type macrophages, interdigitating cells, and reticulocytes. The latter are in particularly close contact with, and infiltrate into, the peripheral portions of the lymphocytes and may influence the differentiation of the thymocytes. Such a regulatory role in the production of thymic lymphocytes has been reported from studies on several vertebrates (*e.g.*, Rappay *et al.*, 1971; Rouse *et al.*, 1979; Duijvestijn and Hoefsmit, 1981). Although the cells of the thymus of *Chimaera* are ultrastructurally compatible with those of other vertebrates, nothing can be said about a functional diversity comparable to that found in vertebrates.

As reported by Fey (1966), the granules of granulocytes from lower vertebrates show a high diversity as to form and electron density. In *Chimaera*, however, there are no difficulties in distinguishing two main groups of granulocytes, eosinophils and heterophils, which can be identified histologically as well as in the electron microscope.

The large spherical granules in the cytoplasm of eosinophils have been identified as lysosomes (Cohn and Wiener, 1963). This has been further supported by Fänge (1968) who showed that the granules concentrate neutral red and suggested that they are supplied with hydrolytic enzymes. The homogeneously electron-dense granules lack a central crystalloid core. This corresponds to results from several lower vertebrates (Kelényi and Németh, 1969; Homma *et al.*, 1984), however, mammals usually

Generally a marginal orientation of the heterochromatin. Some heterophils with pseudopodia (p). Fixation as in Figure 1. Bar: $3 \mu\text{m}$.

FIGURE 7. The orbital organ. Portion of a heterophil. The granules are round or have an irregular outline. Two grades of density occur within most granules. The "empty" region occurs within the dense central region while the outer, less dense area appears granular (arrows). Fixation as in Figure 2. Bar: $1 \mu\text{m}$.

FIGURE 8. Portion of a monocyte from the orbital organ. Solitary characteristic granules (g) supplied with thin halos. Some vacuoles (v) and mitochondria (m). Nucleus (N) with a marked nucleolus (n). Fixation as in Figure 2. Bar: $1 \mu\text{m}$.

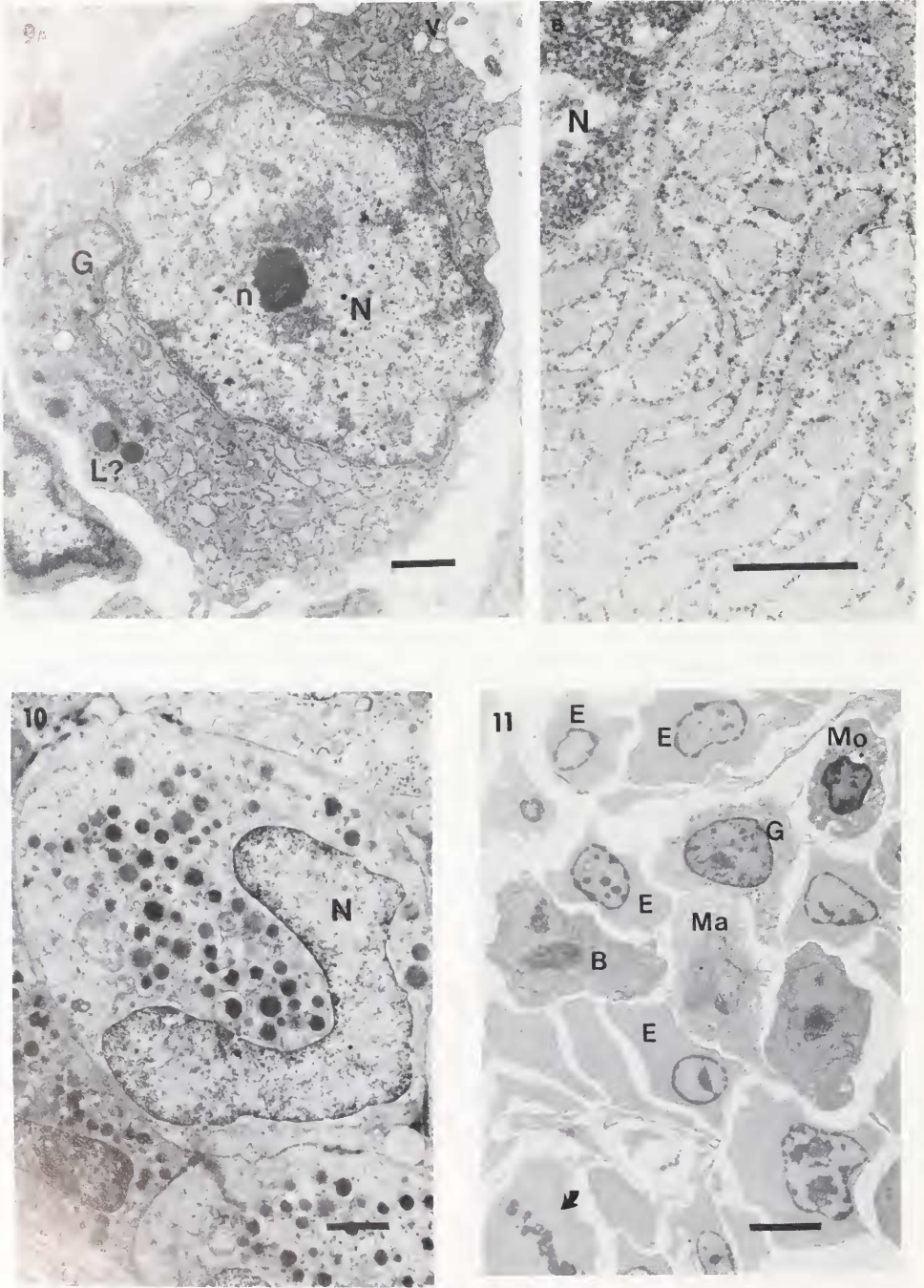


FIGURE 9a. Plasma cell from the orbital organ. Nucleus (N) with a marked nucleolus (n) and scattered heterochromatin. Cytoplasm with the characteristic well-developed rough endoplasmic reticulum, which here forms cisternae. The Golgi complex (G) is prominent. Some lysosome-like bodies (L?) and vacuoles (v) in the cytoplasm.

have a central crystalline structure in their eosinophilic granules (Bargmann and Knoop, 1958; Braunsteiner and Pakesch, 1957).

The heterophils of *Chimaera* show an obvious structural variation, especially in the granules. In the same cell as well as when comparing cells, granules show a marked difference in size, form, and content. Several granules have central regions which show a deviating density, but crystalline inclusions are rare. "Empty" areas often occupy the internal parts of the granules. Such regions, appearing like holes or excavations, may also protrude into the cytoplasm. Similar structures have been reported in amphibians and reptiles and interpreted as signs of an increased interaction between the granules and the cytoplasm (Kelényi and Németh, 1969). Since such "empty" vesicles occur peripherally in the cells, probably in connection with endocytosis, the granules might be interpreted as lysosomes engulfing "empty" endocytotic vesicles. However, there may be another explanation: some of the compact granules have especially electron-dense central regions (arrow in Fig. 7), and a peripheral frame of this dense material often occurs around the central "empty" region (arrow in Fig. 7). This may indicate that the dense material is easily damaged and dissolved during the preparation of the tissue, or that a disappearance of dense material is caused by the electron beam.

The granules of the granulocytes seem to originate at the Golgi region. This is especially obvious for the eosinophils which show a gradual increase in size and density of the granules from the Golgi region towards the periphery of the cells. The great amount of rough endoplasmic reticulum in the peripheral parts of the cells may be correlated to the increased density of the granules and to their suggested increased contents of proteolytic enzymes. The even outlines of the eosinophils give no indication of an endo- or exocytosis and thus no intimation of where or when the enzymes are active.

In the lymphomyeloid tissues of the cranial skeleton of *Chimaera*, granulocytes predominate but often occur near groups of small lymphocytes. The presence of some intermediate forms between lymphocytes and granulocytes points to lymphocyte-like cells as possible stem cells of the granulocytes. The occurrence of blast cells and the high concentration and structural variation of granulocytes in the orbital and suprapalatal tissues indicate that these tissues are main localities for granulocytopoiesis. A granulocytopoietic function seems to be predominant in the cranial lymphomyeloid tissues of *Chimaera* and, like the corresponding lymphomyeloid organs of elasmobranchs, they might be designated "white marrow" or "cartilage marrow" to distinguish them from the mainly haemopoietic red bone marrow of higher vertebrates (Kolmer, 1923).

Since the work by Fagraeus (1948), it has been possible to correlate, in mammals, the formations of antibodies with the occurrence of plasma cells. In the rabbit fish, *Chimaera*, the orbital and suprapalatal tissues hold cells, scattered among the pre-

FIGURE 9b. Higher magnification of a portion of a plasma cell showing numerous cisternae formed by the rough endoplasmic reticulum and their moderately electron-dense interior. Nucleus (N). Fixation as in Figure 1. Bar in 9a: 1 μm . Bar in 9b: 0.5 μm .

FIGURE 10. Heterophil from the suprapalatal region. The cytoplasmic granules are comparatively small and spherical and lack central "empty" zones. The whole cell appears faintly electron-dense. Horseshoe-shaped nucleus (N). Fixation as in Figure 2. Bar: 1 μm .

FIGURE 11. A survey of cells from the spleen. Erythrocytes (E) predominate. Other cell types: macrophage (Ma), monocyte-like cell (Mo), granulocyte (G) and blast cell (B). At arrow a mitosis. Fixation as in Figure 1. Bar: 5 μm .

dominating granulocytes and which have the ultrastructure of plasma cells. Morphologically they deviate in two respects from the plasma cells of higher vertebrates: their heterochromatin does not show an arrangement of radial strings and their rough endoplasmic reticulum forms a number of cistern-like structures. Otherwise, the rough endoplasmic reticulum shows an abundance and an arrangement that strongly suggest these cells to be plasma cells with their comprehensive protein synthesis. As shown by Fänge and Sundell (1969), the blood plasma of *Chimaera* contains two or three protein fractions migrating towards the cathode like the gammaglobulins of higher vertebrates. In another holocephalan fish, *Callorhynchus callorhynchus*, Sánchez *et al.* (1980) showed the presence of an IgM-like hemagglutinin. This macromolecule has been further studied by Garrido and De Ioannes (1981) by using electron microscopy. They suggested a pentameric structure similar to that reported for human IgM. However, the identification of antibodies in *Chimaera monstrosa* as well as of cells performing an antibody synthesis must await further research.

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